The Biological Mechanisms of Air Ion Action

I. 5-Hydroxytryptamine as the endogenous mediator of positive air ion effects on the mammalian trachea

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ABSTRACT Intravenous administration of 5-hydroxytryptamine to rabbits and guinea pigs is shown to bring about changes very similar to those produced by (+) air ions, including (1) decreased ciliary rate, (2) contraction of the posterior tracheal wall, (3) exaggerated response of the tracheal mucosa to trauma, (4) marked vasoconstriction in the tracheal wall, and (5) increased respiratory rate. These effects are reversed by (−) air ions. Iproniazid, which raises 5-hydroxytryptamine levels in the animal by blocking monamine oxidase, produces similar but non-reversible effects. Reserpine, which depletes 5-hydroxytryptamine in the animal, causes changes that resemble those produced by (−) air ions, including (1) increased ciliary rate, (2) relaxed posterior sulcus, (3) hyperemia of the tracheal mucosa, (4) lowered respiratory rate, and (5) increased volume and rate of mucus flow.

On the basis of these facts, the hypothesis is advanced that (+) air ion effects are mediated by the release of free 5-hydroxytryptamine, while (−) air ion effects depend on the ability of (−) ions to accelerate the enzymatic oxidation of 5-hydroxytryptamine.

INTRODUCTION

Within the past 2 years, exposure of the mouse, rat, guinea pig, rabbit, and monkey trachea to air ions has been shown to produce a number of characteristic and highly reproducible effects (1–3). These effects include:

(+) *Ions*	(−) *Ions*
1. Decreased ciliary rate	1. Increased ciliary rate
2. Contraction of posterior tracheal wall	2. Reversal of (+) ion induced contraction of tracheal wall

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This research was supported by contracts with: (1) Atomic Energy Commission and (2) United States Air Force (A.F. 49 (638)-669) monitored by the Air Force Office of Scientific Research of the Air Research and Development Command, and by a grant from the Committee on Research of the University of California.

The opinions expressed herein are not necessarily those of the Navy Department or of the Air Force.

Received for publication, May 29, 1959.

3. Exaggerated response of tracheal mucosa to mild mechanical trauma	3. No response of tracheal mucosa to mild mechanical trauma
4. Vasoconstriction in tracheal wall	4. Normal vascularity in tracheal wall
5. Increased respiratory rate	5. Decreased respiratory rate

The first three effects are seen in the extirpated tracheal strip; all five effects are seen in the living tracheotomized animal.

While a partial explanation for negative ion action is implied by the recently discovered ability of negative ions *in vitro* to accelerate metabolic reactions catalyzed by cytochrome oxidase (4), positive air ions exert no measurable effect on these reactions. Positive air ions evidently act through a different mechanism.

Recently we have been impressed by the similarity between the observed effects of positive air ions and some of the general physiological effects attributed to 5-hydroxytryptamine (5-HT, serotonin). In particular, the reported ability of 5-hydroxytryptamine to cause smooth muscle contraction, vasoconstriction, and increased respiratory rates (5), seems to resemble the last three effects listed above for positive ions.

With a view toward uncovering any possible relationship between positive air ions and 5-hydroxytryptamine, the following experiments were undertaken.

In addition to 5-hydroxytryptamine itself, use was made of reserpine and iproniazid. The latter two compounds have been shown capable of lowering and raising, respectively, 5-hydroxytryptamine levels in mammalian tissues (6, 7).

Materials and Methods

The drugs used in these experiments consisted of a 1 per cent solution of 5-hydroxytryptamine (Sigma), ampoules of reserpine (Ciba) containing 5 mg./2 ml., and a solution of iproniazid (Roche) containing 50 mg./3 ml.

For the *in vivo* studies, rabbits anesthetized with sodium pentobarbital were tracheotomized according to a technique already described (2) and given one or more of the drugs intravenously as indicated by the figures.

For the *in vitro* studies, rabbits and guinea pigs were pretreated with one of the three drugs, then sacrificed, and their tracheas removed according to a technique already described (1). The pretreatment consisted of (1) intracardial injection of 10 mg. 5-hydroxytryptamine just before sacrifice, (2) intraperitoneal injection of 50 to 100 mg. iproniazid 20 hours before sacrifice, or (3) intraperitoneal injection of 5 mg. reserpine 20 hours before sacrifice.

The humid-air exposure chamber and the ion source were the same as described elsewhere (2). When the extirpated trachea was exposed to air ions, a portion of the trachea was retained in an un-ionized humid chamber as a control.

EXPERIMENTAL RESULTS

I. Effects of 5-Hydroxytryptamine, Iproniazid, and Reserpine on Tracheal Function

A. 5-HYDROXYTRYPTAMINE Intravenous administration of 5-hydroxytryptamine to a living tracheotomized animal caused (1) rapid reduction of the ciliary rate (Fig. 1A), or complete cessation of ciliary activity, (2) contraction of the posterior tracheal wall, (3) an enhanced vulnerability of the

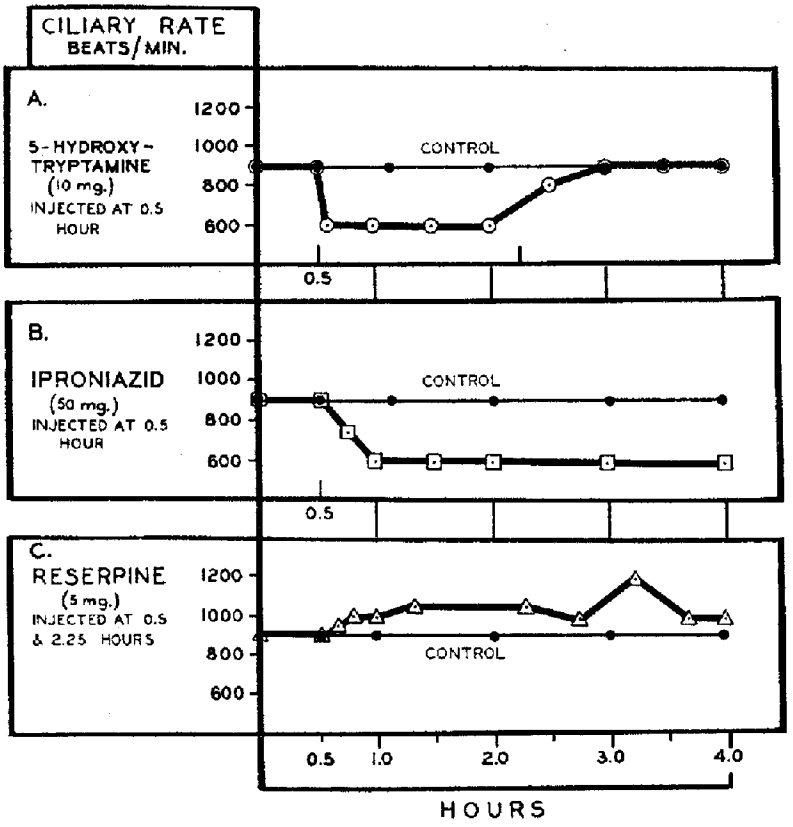

FIGURE 1. Effect of intravenous injection of 5-hydroxytryptamine, iproniazid, and reserpine on the ciliary rate of the living rabbit trachea.

tracheal mucosa to trauma so pronounced that the slightest touch with a blunt probe instantly produced a conspicuous ecchymotic disc, (4) abrupt blanching of the tracheal mucosa, and (5) acceleration of the respiratory rate.

When 5-hydroxytryptamine was administered intracardially to an animal just before sacrifice, the extirpated trachea had a characteristic "bloodless"

appearance, a contracted posterior sulcus, and a depressed or absent ciliary beat.

When large doses of 5-hydroxytryptamine were given, ciliary activity remained permanently impaired. With smaller doses, the ciliary beat gradually returned to normal after an hour or longer (Fig. 1A).

B. IPRONIAZID In general, iproniazid produced the same effects on the trachea as 5-hydroxytryptamine, only at a slower rate and quite irreversibly. There were no spontaneous returns to normal ciliary activity in this group. Drying of the tracheal mucosa was regularly noted (Fig. 1B).

C. RESERPINE Intravenous administration of reserpine to a living tracheotomized animal (1) raised the ciliary rate (Fig. 1C), (2) relaxed the posterior sulcus, (3) caused marked hyperemia of the tracheal mucosa, (4) depressed the respiratory rate, and (5) raised the volume and rate of mucus flow.

When an animal was pretreated with reserpine before sacrifice, the extirpated trachea was found engorged with blood, the posterior sulcus relaxed, and the ciliary rate greatly accelerated—in some cases as high as 1250 beats/minute (normal ciliary rate = 850 to 900 beats/minute).

II. Effects of Air Ions on the Tracheal Function of the Drug Pretreated Animals

A. 5-HYDROXYTRYPTAMINE AND AIR IONS The rate of recovery of animals pretreated with 5-hydroxytryptamine could be greatly accelerated by exposing the tissue to (−) air ions (Fig. 2).

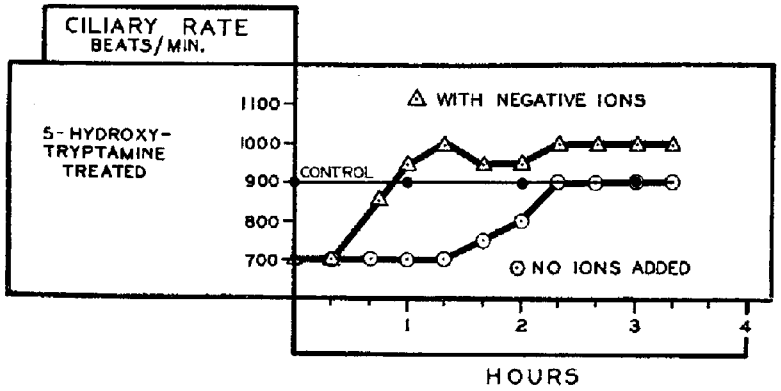

FIGURE 2. Effect of negative air ions on the ciliary rate of the extirpated trachea of a rabbit pretreated with 5-hydroxytryptamine.

Since 5-hydroxytryptamine produces the same effects as (+) air ions, exposure of these tissues to (+) ions either had an additive effect or no effect at all.

B. IPRONIAZID AND AIR IONS Prolonged exposure of the tracheas of iproniazid-treated animals to (−) air ions failed to restore normal tracheal functioning (Fig. 3B).

One again, (+) air ions either had an additive effect or no effect at all.

C. RESERPINE AND AIR IONS Prolonged exposure of the tracheas of reserpine-treated animals to (+) air ions failed to bring the enhanced ciliary rate down to normal levels (Fig. 3A).

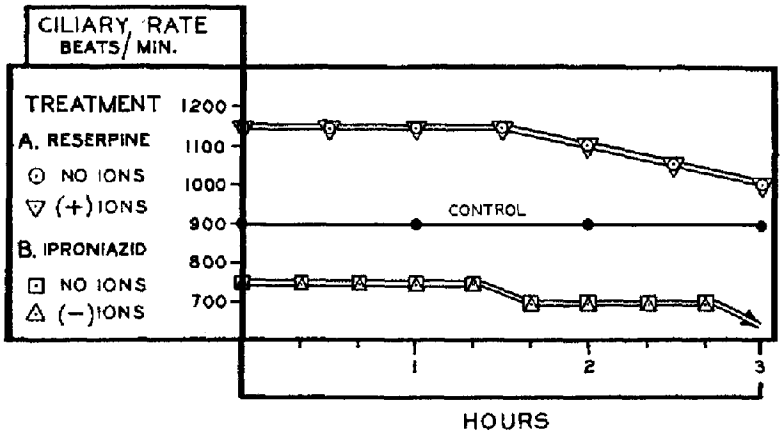

FIGURE 3. Effect of air ions on the ciliary rates of the extirpated tracheas of rabbits pretreated with reserpine and iproniazid.

When the extirpated trachea is exposed to any agent that accelerates ciliary activity, a gradual decline in this enhanced rate occurs after 2 to 3 hours. This decline seems to represent an exhaustion of nutrients in the abnormally active epithelial cells, as it is not observed at all in the living tracheotomized animal, or as rapidly in the extirpated trachea control. None of the other reserpine-induced effects described in I-C was in any way altered by (+) air ions.

Since the trachea of the reserpine-treated animal already shows all the effects produced by (−) air ions, exposure of such a tissue to (−) ions has no detectable effect.

DISCUSSION

In previous experiments we were able to establish that negatively ionized oxygen and positively ionized carbon dioxide are the components of ionized air actually responsible for the various biological effects ascribed to "negative air ions" and "positive air ions" (10). We can now postulate the biological mechanisms by which these components produce their characteristic effects on the mammalian trachea.

All the tracheal effects attributed to (+) air ions can be produced by the intravenous injection of 5-hydroxytryptamine. Like (+) ion effects, the 5-HT effects can be reversed by treatment with (−) air ions. On the basis of these facts, it seems reasonable to postulate that (+) air ions are "serotonin releasers," and that a local accumulation of 5-HT in the trachea is the immediate cause of (+) ion effects (Fig. 4B). Normal metabolism of 5-HT is shown in Fig. 4A.

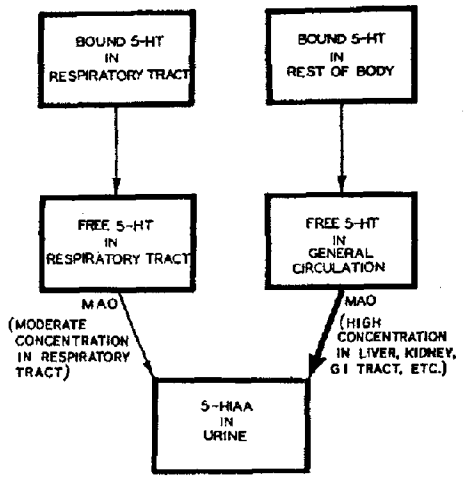

FIGURE 4. Schematic presentation of 5-hydroxytryptamine metabolism and the effects of air ions, reserpine, and iproniazid.
FIGURE 4A. Normal metabolism of 5-hydroxytryptamine.

It can further be postulated that (−) air ions reverse (+) ion effects by speeding up the rate at which free 5-HT is oxidized (Fig. 4C). Like other oxidase systems, monamine oxidase is thought to consist of a dehydrogenase linked to a respiratory chain which may include cytochromes or flavins (8). (−) Ions have been shown to have a direct action on cytochrome oxidase and to accelerate the cytochrome-linked conversion of succinate to fumarate (4). This same action might also accelerate a cytochrome-linked oxidation of 5-HT.

The experiments with reserpine and iproniazid provide indirect confirmation of this hypothesis.

Reserpine is believed to cause 5-HT to be momentarily released and then rapidly destroyed by monamine oxidase (6), so that the tissues are quickly depleted of 5-HT (Fig. 4D). If our hypothesis is correct, reserpine would produce a condition in the trachea resembling that induced by (−) air ions. Moreover, one would expect (+) air ions to be unable to produce their characteristic effects on a reserpine-treated animal, since the 5-HT necessary for

(+) ion action is lacking. Both these expectations have been realized experimentally.

In contrast, iproniazid blocks the enzyme responsible for metabolizing

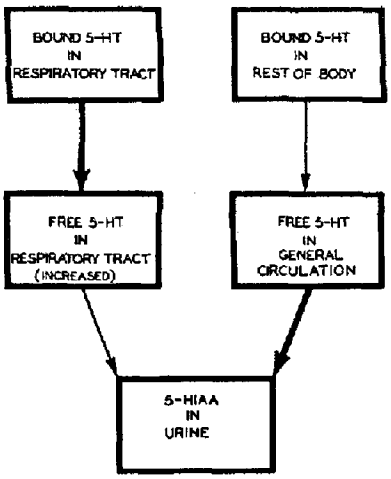

FIGURE 4B. Hypothesized action of (+) air ions.

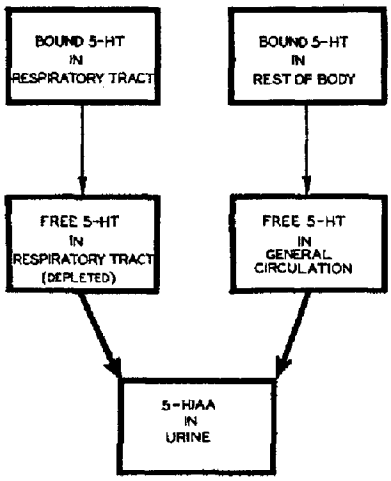

FIGURE 4C. Hypothesized action of (−) air ions.

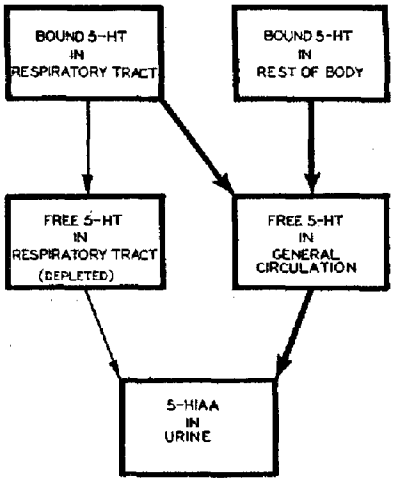

FIGURE 4D. Action of reserpine.

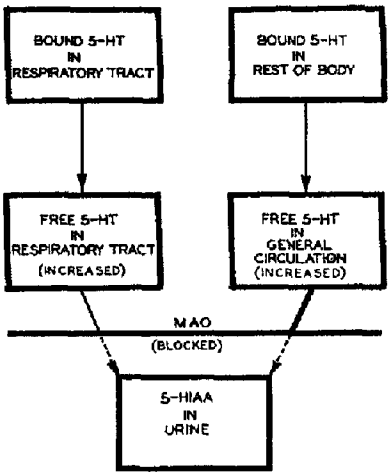

FIGURE 4E. Action of iproniazid.

5-HT (9), so that an accumulation of free 5-HT develops (Fig. 4E). One would expect an iproniazid-treated animal to display tracheal effects resembling those produced by (+) air ions and to resist the normal action of (−) ions in reversing these effects. Again both these expectations have been experimentally confirmed.

Suggestive though it is, the evidence so far is only circumstantial. Before the hypothesis can be considered proven, it will be necessary to show directly that (+) air ions release 5-HT, and that (−) air ions accelerate its metabolism. Such experiments are currently in progress.

Whether or not our hypothesis is correct, the facts remain that (1) all the tracheal effects produced by (+) or (−) air ions can be duplicated by drugs that operate through the release or metabolic blocking of 5-hydroxytryptamine, (2) by using these drugs, it is possible to prevent excesses of (+) or (−) air ions from exerting their usual effects on the respiratory tract, and (3) by means of air ions, it is possible to reproduce the effects of reserpine and iproniazid on the respiratory tract without the untoward systemic effects of these drugs.

We wish to express our gratitude to John C. Beckett for his helpful advice in solving problems of ion generation and measurement.

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